THE THERMAL DEATH POINT OF THE SPORES OF BACILLUS BOTULINUS IN CANNED FOODS

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I reported [1] the results of an investigation of the resistance of the spores of B. botulinus [2] to heat and the effects of several variable conditions which influence their death. It was shown that the thermal death point is markedly influenced by the hydrogen-ion concentration. It was also shown that the destruction of the spore is a gradual process, not an instantaneous killing; that is, the spores are progressively injured and finally destroyed. It was demonstrated that spores of different strains vary considerably in their heat resistance; that the resistance is influenced by the age of the spores; that young spores about 1 month old have the highest thermal resistance; that increasing the concentration of sodium chloride decreases the resistance; and that the larger the number of spores the higher the temperature and longer the period of exposure required to kill them. The relative importance of these factors was also shown.

On the basis of these facts I proceeded to a study of the heat resistance of the spores of B. botulinus in commercially canned foods. Thirty-six varieties of standard brands of foods on the American market were tested.

Technic

The can liquor was removed with aseptic precautions. In cans in which there was no free liquor the juice was expressed through sterile gauze into a sterile container. The spore suspension was prepared by growing B. botulinus in sheep's brain medium for one month and straining through sterile gauze. Nine parts of the food fluid to be tested was mixed with one part of the spore suspension, and 1 c c of this mixture was pipetted into a tube, 10 mm. inside diameter, 12 mm. outside diameter and 30 cm. long. A series of such tubes were prepared. Special tubes were used, care being required in their manufacture and selection to insure a uniform quality of glass and a uniform thickness of wall to obviate experimental errors arising from the influence of these factors on the rate of heat penetration. The change

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[1] Weiss, H.: The Heat Resistance of Spores with Special Reference to the Spores of B. botulinus, Jour. Infect. Dis., 28, p. 70.

[2] Committee of Soc. Am. Bact. (Jour. Bacteriol., 1920, 5, p. 222) recommends that this organism be called Clostridium botulinum.

In Hygienic Laboratory Bull. No. 121, Sept., 1920, E. M. A. Enlows, Jensen classifies this organism as Botulobacillus botulinus.

in the P_H value due to the glass was determined and was found to be negligible under our experimental conditions. The tubes were heated in a Bunsen flame to within 2 cm. of the surface of the fluid in order to kill all spores which might have been deposited on the glass. When these had cooled, they were sealed in the flame submerged to a depth of 12 cm. in a DeKhotinsky oil bath and exposed to the action of heat. A series of tubes was prepared in this manner for exposure at a given temperature, and at certain intervals a tube was removed, opened, and 10 c c of freshly heated glucose agar added, care being taken to mix thoroughly the spore suspension throughout the agar. The tubes were then incubated at 37.5 C. for at least 3 months. The long period of incubation was necessary in order to determine delayed germination of injured spores.[1]

The experiment was planned to determine the thermal death point of the spores of B. botulinus under laboratory conditions arranged to approximate as nearly as practicable the conditions in the can. The food juice was tested immediately after its removal from the can, its hydrogen-ion concentration determined and a note made of the consistency of the liquor obtained. This consistency varied from a colored or almost colorless watery solution in some products, such as Brussels sprouts, string beans, peas, asparagus, etc., to an opaque, heavy, almost gelatinous mixture in others, such as succotash, pork and beans, chili con carne, etc. The influence of the physical constitution of the food juice will be indicated later.

One strain of B. botulinus (our laboratory strain 15) was used in all the exposures. It was originally isolated by Edmondson from asparagus salad, which had caused the death of 4 persons at Boise, Idaho, in January, 1919. This strain was chosen from 16 because it produces spores of a higher resistance than any other strain of unquestionable origin; its identity is certain; it is an active gas producer on glucose mediums and produces a toxin of high virulence.

It has been shown [1] that the thermal resistance of a spore suspension varies with the age of the spore, the greatest resistance being found to exist when the spore is about 1 month old. In order to keep this factor constant spores of this age were employed.

The degree of heat and time of exposure necessary to accomplish sterility vary with the number of spores present in the suspension. The greater the number, the more heat is required. The initial spore suspension as grown contains approximately 15,000,000 spores per c c. One part of this suspension strained through sterile gauze was diluted with 9 parts of the food liquor. After dilution each tube contained

1,500,000 spores in 1 c c of volume. I have chosen 1,500,000 spores per c c as a standard concentration, believing that that is far greater than the number of spores that can possibly be present as a contamination in any canned product.

RESULTS

The results indicate that the size of the container being constant, there are at least two primary factors that determine the length of exposure and degree of heat required to accomplish sterility: (1) the hydrogen-ion concentration, and (2) the physical character or consistency of the food.

I have shown [1] that the spores of B. botulinus show their greatest thermal resistance at the point of neutrality or very near that point when pure acids and alkali are used, and that the thermal resistance rapidly diminishes as the hydrogen-ion concentration increases or decreases from that point. The results with the juices of canned food products substantiate this conclusion (see diagram).

Analysis of the results show that the spores of B. botulinus are killed in all food juices (such as the 11 fruit products given in the table) having a p_H value between 2.1 and 3.85 in 50 minutes or less at 100 C. The greater number of these fruit juices require only 30 minutes or less. Less acid foods having a p_H of 4.22 and 4.4 require 60 to 90 minutes at the same temperature. Beets, asparagus, wax beans, peas, squash and sweet potatoes having p_H values of 5.13 to 5.36 require an exposure of 90 to 120 minutes at 100 C. to kill the spores of B. botulinus. Pork and beans, red kidney beans, lima beans, chile con carne, succotash and sweet corn with p_H values of 5.69 to 6.21 require 150 to 180 minutes at 100 C. to accomplish the same result.

Other factors being equal, foods having an alkaline reaction as well as those having an acid reaction, require a shorter period of exposure to kill the spores of B. botulinus than food which has a neutral reaction. For example, food number 21 (hominy) with a p_H value of 7.4 required only 90 minutes at 100 C., while food number 22 (shrimp) with a p_H value of 7.00 required 150 minutes or almost twice that period of exposure. The results are shown graphically in the accompanying tabular diagram (see diagram).

Another fact that is apparent is that all the fruits and fruit products tested form a group showing the highest acidity. The p_H values of the products in this group fall between 2.1 and 3.85, and the maximum period of exposure required at 100 C. is 50 minutes. The second group

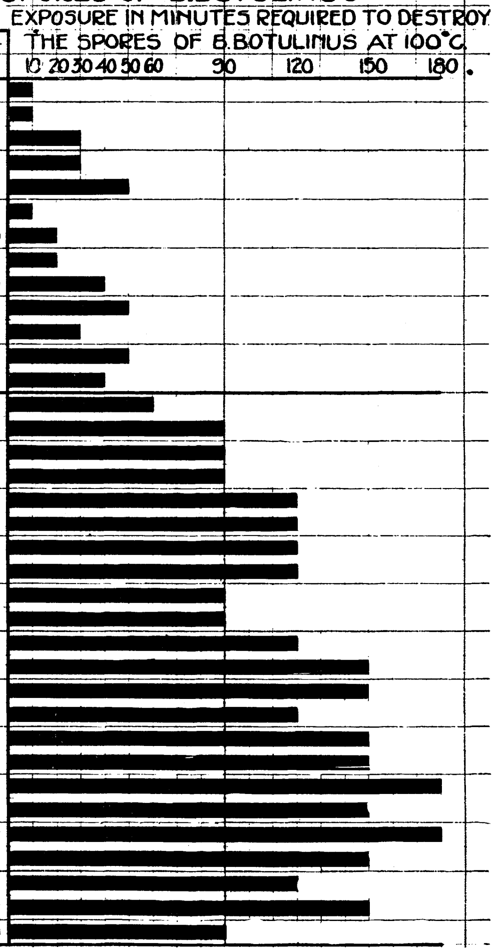
TABLE 1

Thermal Death Point of the Spores of B. botulinus in Canned Goods

No.	Food	Brand	H	P_H	Time, Minutes—100 C.												Time, Minutes—105 C.								Time, Minutes—110 C					
					0	10	20	30	40	50	60	90	120	150	180	210	0	10	20	30	40	50	60	70	0	5	10	15	20	25
14	Apples	Boyers	5.8×10^{-4}	3.24	+	+	+	+	+	—	—	—	—	—	—	—	+	+	—	—	—	—	—	..	+	+	—	—	—	—
36	Apricots	Silver Bar	2.4×10^{-4}	3.62	+	+	+	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	..	+	—	—	—	—	—
3	Asparagus	Bonvallet	7.0×10^{-6}	5.16	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	+	—	—	—	..	+	+	+	—	—	—
10	Beans, lima	Luxury	1.7×10^{-6}	5.77	+	+	+	+	+	+	+	+	+	+	—	—	+	+	+	+	+	—	—	..	+	+	—	—	—	—
2	Beans, pork and plain	Hart	2.1×10^{-6}	5.69	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+	—	—	—	..	+	+	+	+	—	—
13	Beans, pork and tomato sauce	Hart	4.0×10^{-6}	5.40	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+	—	—	—	..	+	+	+	+	—	—
12	Beans, red kidney	Hart	2.0×10^{-6}	5.70	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+	—	—	—	..	+	+	+	—	—	—
6	Beans, string	Hart	4.0×10^{-6}	5.40	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	—	—	—	—	..	+	+	+	—	—	—
32	Beans, wax	New York Canners	6.0×10^{-6}	5.22	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	+	—	—	—	..	+	+	—	—	—	—
5	Beets	Rose Carnival	7.5×10^{-6}	5.13	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	+	—	—	—	..	+	+	+	—	—	—
15	Blackberries	Brunswick	2.8×10^{-4}	3.55	+	+	+	+	+	—	—	—	—	—	—	—	+	+	—	—	—	—	—	..	+	+	—	—	—	—
19	Blueberries	National Canners	4.0×10^{-4}	3.40	+	+	+	+	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	..	+	+	—	—	—	—
35	Brussels sprouts	Del-Monte	3.5×10^{-6}	5.46	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	—	—	—	—	..	+	+	—	—	—	—
37	Carrots	Diamond "A"	9.0×10^{-6}	5.05	+	+	+	+	+	+	+	—	—	—	—	—	+	+	+	—	—	—	—	..	+	+	—	—	—	—
27	Cherries, pitted red	New York Canners	5.0×10^{-4}	3.80	+	+	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	..	+	+	—	—	—	—
9	Chile con carne	Walkers	1.5×10^{-6}	5.82	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+	+	+	—	..	+	+	+	—	—	—
18	Cider	National Canners	5.0×10^{-4}	3.30	+	+	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	..	+	—	—	—	—	—
8	Clam chowder	Red Label, S. S. P.	5.5×10^{-7}	6.26	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	+	—	—	—	..	+	+	+	+	—	—
7	Corn, sweet	New York Canners	6.2×10^{-7}	6.21	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+	—	—	—	..	+	+	—	+	—	—
28	Gooseberries	New York Canners	1.0×10^{-3}	3.00	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	..	+	—	—	—	—	—
21	Hominy	National Canners	4.0×10^{-8}	7.40	+	+	+	+	+	+	+	—	—	—	—	—	+	+	—	—	—	—	—	..	+	+	—	—	—	—
16	Peaches	Brunswick	2.0×10^{-4}	3.70	+	+	+	+	+	—	—	—	—	—	—	—	+	+	—	—	—	—	—	..	+	+	+	—	—	—
26	Pears, Bartlett	New York Canners	1.4×10^{-4}	3.85	+	+	+	+	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	..	+	+	—	—	—	—
4	Peas, sweet wrinkled	Cazenovia	5.0×10^{-6}	5.30	+	+	+	+	+	+	+	+	—	—	—	—	+	+	+	+	—	—	—	..	+	+	+	+	—	—
29	Plums, egg	New York Canners	8.0×10^{-3}	2.10	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	..	+	—	—	—	—	—
11	Pumpkin	Reelyrite	6.0×10^{-5}	4.22	+	+	+	+	+	+	+	—	—	—	—	—	+	+	+	—	—	—	—	..	+	+	—	—	—	—
25	Raspberries, red	New York Canners	7.0×10^{-4}	3.16	+	+	+	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	..	+	+	—	—	—	—
34	Sauerkraut	Del-Monte	5.5×10^{-4}	3.26	+	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	..	+	—	—	—	—	—
22	Shrimp	Sea Bird	1.0×10^{-7}	7.00	+	+	+	+	+	+	+	+	+	—	—	—	+	+	+	+	—	—	—	..	+	+	+	—	—	—
1	Spinach	Reelyrite	4.0×10^{-6}	5.40	+	+	+	+	+	+	+	+	+	—	—	—	+	+	—	+	—	—	—	..	+	+	+	—	—	—
30	Squash	New York Canners	5.0×10^{-6}	5.30	+	+	+	+	+	+	+	—	—	—	—	—	+	+	+	—	—	—	—	..	+	+	—	—	—	—
17	Strawberries	National Canners	6.0×10^{-4}	3.22	+	+	+	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	..	+	—	—	—	—	—
31	Succotash	New York Canners	1.0×10^{-6}	6.00	+	+	+	+	+	+	+	+	+	+	—	—	+	+	+	+	+	+	+	—	+	+	+	+	—	—
33	Sweet potatoes	Del-Monte	4.4×10^{-6}	5.36	+	+	+	+	+	+	+	—	—	—	—	—	+	+	+	—	—	—	—	..	+	+	+	—	—	—
20	Tomatoes	S. S. Pierce	4.0×10^{-5}	4.40	+	+	+	+	+	+	—	—	—	—	—	—	+	+	+	+	—	—	—	..	+	+	+	—	—	—
38	Turnips	Diamond "A"	8.0×10^{-5}	4.10	+	+	+	+	+	+	—	—	—	—	—	—	+	+	—	—	—	—	—	..	+	+	—	—	—	—

TABULAR DIAGRAM SHOWING THE EFFECT OF THE pH VALUE OF VARIOUS CANNED FOODS ON THE THERMAL RESISTANCE OF THE SPORES OF B. BOTULINUS —

	No.	Food	pH
GROUP I	29	Egg Plums	2.10
	28	Gooseberries	3.00
	25	Raspberries	3.16
	17	Strawberries	3.22
	14	Apples	3.24
	34	Sauerkraut	3.26
	27	Cherries	3.30
	18	Cider	3.30
	19	Blueberries	3.40
	15	Blackberries	3.55
	36	Apricots	3.62
	16	Peaches	3.70
	26	Bartlett Pears	3.85
GROUP II	38	Turnips	4.10
	11	Pumpkin	4.22
	20	Tomatoes	4.40
	37	Carrots	5.05
	5	Beets	5.13
	3	Asparagus	5.16
	32	Wax Beans	5.22
	4	Sweet Wrinkled Peas	5.30
	30	Squash	5.30
	33	Sweet Potatoes	5.36
	6	String Beans	5.40
	13	Pork & Beans-Tom. S.	5.40
	1	Spinach	5.40
	35	Brussels Sprouts	5.46
	2	Pork & Beans-Plain S.	5.69
	12	Red Kidney Beans	5.70
	10	Lima Beans	5.77
	9	Chile Con Carne	5.82
	31	Succotash	6.00
	7	Sweet Corn	6.21
	8	Clam Chowder	6.26
	22	Shrimp	7.00
	21	Hominy	7.40



with p_H values ranging from 4.1 to 7.4 requires an exposure of 90 minutes to 180 minutes at the same temperature with the single exception of turnips which require 60 minutes, their p_H value being 4.0, thus most nearly approaching the hydrogen-ion concentration in the fruit group. This second group includes all the vegetable products to the complete exclusion of all the fruit products.

It is evident from the diagram that in the first group a number of products of almost equal p_H value show differences of 10 and 20 minutes in the exposure required to kill the spores. This difference is apparently due in most cases to the concentration of syrup present in the product, the greater the concentration of syrup the longer the period of exposure required.

In the second group, 3 products—string beans, pork and beans in tomato sauce and spinach—with similar p_H value, show a variation of 30 minutes in the exposure required at 100 C. to kill the spores. This is apparently due to the consistency of the foods in question. Of these 3 foods having a p_H value of 5.40, string beans, which have a loose consistency and are completely bathed in liquor, require 120 minutes at 100 C., while pork and beans in tomato sauce and spinach require 30 minutes longer, due to the fact that these products are of a heavier or thicker consistency. Similarly, the liquor of Brussels sprouts with a p_H value of 5.46 requires an exposure of 120 minutes at 100 C., while the liquor of pork and beans in plain sauce and red kidney beans, which have only a slightly higher p_H value (5.69 and 5.70), requires 150 minutes at the same temperature. Succotash, sweet corn and clam chowder, having p_H values of 6.00, 6.21 and 6.26, are more fluid in the order named, and as a result require 180, 150 and 120 minutes exposure, respectively.

Another important factor in the sterilization of canned foods is the time required for the heat to penetrate to the center of the can. This phase has been fully treated by Bigelow, Bohart, Richardson and Ball.[2] The effective temperature and periods of heating given in this paper are exclusive of the period of penetration which varies with the particular food and the size of the can. For practical purposes the period of exposure required for any food product will be expressed by the period given plus a factor depending on the size of the can, the specific coefficient of heat penetration, the retort technic, etc.

[2] Bull. 16-L, Research Laboratory, National Canners Association, Washington, D. C.

SUMMARY

The thermal death point of the spores of B. botulinus in the juices of 36 varieties of canned food on the American market has been determined.

The thermal death point varies with the hydrogen-ion concentration of the particular food in question. The more acid foods, such as canned fruits, require a maximum of 50 minutes at 100 C., 30 minutes at 105 C. and 15 minutes at 110 C.; a majority of this group require much shorter exposures at the temperatures given. Thus, 8 of the 11 food products in this first group require 30 minutes or less at 100 C. The vegetable products, which are less acid and more nearly approach the neutral reaction, require from 90 to 180 minutes' exposure at 100 C., 30 to 70 minutes' exposure at 105 C., and 10 to 20 minutes' exposure at 110 C.

The thermal death point also depends on the consistency of the particular food, the more fluid products requiring a shorter period of exposure at a given temperature than the less fluid ones.

The thermal death point is also influenced by the presence and concentration of syrup. The heavier the syrup, the longer the period of exposure required at any one temperature.

The temperatures and times of exposure given in this paper must not be directly applied to practical canning, for these factors vary not only with the nature and the p_H value of the food in question, but also with the size of the can, the size and compactness of the cook and the retort technic. Furthermore, a factor of safety should be added to insure sterilization under practical commercial conditions.